Supplemental Figures

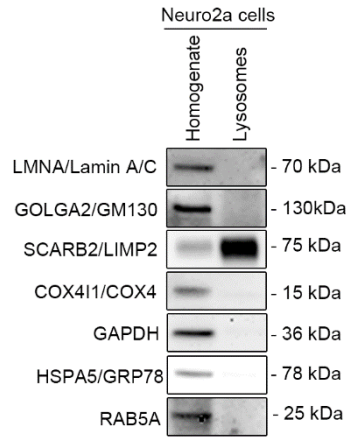

Figure S1. Immunoblotting of lysosomes isolated from Neuro2a cells. Neuro2a cell homogenates and lysosomes isolated from Neuro2a cell homogenates were subjected to immunoblotting using antibodies against nucleus marker LMNA/lamin A/C, Golgi apparatus marker GOLGA2/GM130, lysosomal marker SCARB2/LIMP2, mitochondrion marker COX4I1/COX4, cytosolic marker GAPDH, endoplasmic reticulum marker HSPA5/GRP78 (anti-KDEL antibody) and early endosome marker RAB5A.

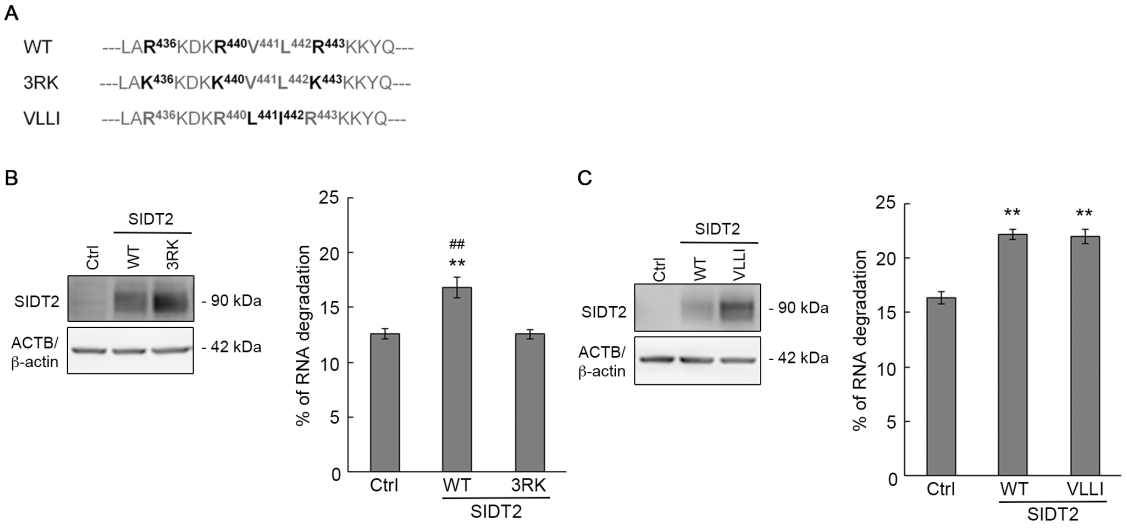

Figure S2. Requirement of arginine residues of SIDT2-CD for SIDT2-mediated RNA degradation in cells. (**A**) Partial amino acid sequence of WT SIDT2 and mutants that are used in this study. (**B**, left panel) Neuro2a cells seeded in 24-well culture plates were transfected with pCI-neo (control) or WT or 3RK SIDT2 and after 28 h, cells were harvested with lysis buffer and western blotting was performed using anti-SIDT2 and anti-ACTB/β-actin antibodies. (**B**, right panel) Degradation of endogenous RNA was assessed in control and WT or 3RK SIDT2-overexpressing Neuro2a cells by pulse-chase analysis. Cells were labeled with [^{3}H]-uridine 24 h post-transfection. Radioactivity was expressed as a percentage of degraded RNA. **, $P < 0.01$ vs Ctrl; ##, $P < 0.01$ vs 3RK (n = 4). (**C**, left panel) Neuro2a cells seeded in 24-well culture plates were transfected with pCI-neo (control) or WT or VLLI SIDT2 and after 28 h, cells were harvested with lysis buffer and western blotting was performed using anti-SIDT2 and anti-ACTB/β-actin antibodies. (**C**, right panel) Degradation of endogenous RNA was assessed in control and WT or 3RK SIDT2-overexpressing Neuro2a cells by pulse-chase analysis. Cells were labeled with [^{3}H]-uridine 24 h post-transfection. Radioactivity was expressed as a percentage of degraded RNA. **, $P < 0.01$ vs Ctrl (n = 4).

A

TAATACGACTCACTATAGGGAGACCCAAGCTTGGTACCGAGCTCGGATCCCGCCATGGCG
ACCCTGGAAAAGCTGATGAAGGCCTTCGAGTCCCTCAAGTCCTTC(CAG)nCCGCCACCG
CCGCCGCCGCCGCCGCCGCCGCCTCCTCAGCTTCCTCAGCCGCCGCCGCAGGCACAG
CCGCTGCTGCCTCAGCCGCAGCCGCCCCCGCCGCCGCCCCCGCCGCCACCCGGCCCGGCT
GTGGCTGAGGAGCCGCTGGATCCAGACTACAAAGACGATGACGACAAGTGAGATATC

B

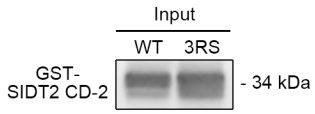

Figure S3. *HTTex1* RNA and GST-CD-2 proteins used in the affinity-isolation assay. (**A**) Sequence of the template DNA used for *in vitro* transcription. The pcDNA3-*HTT* plasmids were cut with EcoRV and used as template for *in vitro* transcription. Sequences of the T7 promoter, start codon, CAG repeats (n = 22 or 110), stop codon and EcoRV site are shown in purple, green, red, orange and blue letters, respectively. The sequence that is predicted to be transcribed by T7 polymerase is underlined. (**B**) Input GST-CD-2 proteins used in the affinity-isolation assay (Figure 6A,B) were analyzed by western blotting using anti-GST antibody.

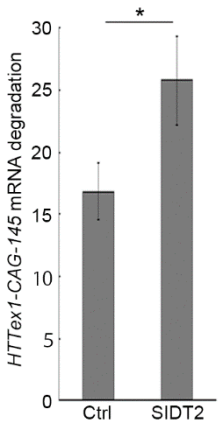

Figure S4. SIDT2 overexpression promotes degradation of *HTTex1-CAG-145* mRNA in cells. Degradation of *HTTex1-CAG-145* mRNA was assessed in control and SIDT2-overexpressing Neuro2a cells and quantification was performed by qPCR. RNA levels were expressed as percentage of degraded RNA. *, $P < 0.01$ (n = 3).